Acta Medica Okayama

Volume 33, *Issue* 5 1979 *Article* 2

OCTOBER 1979

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The mechanism of the release of hepatic enzymes in various liver diseases. 1. Alterations in cytoplasmic and mitochondrial enzyme activities in serum.*

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Abstract

Serum glutamic oxaloacetic transaminase (GOT), mitochondrial GOT (GOTm), glutamic-pyruvic transaminase (GPT) and glutamate dehydrogenase activities were determined in 43 healthy controls and in 280 cases of liver diseases. A simplified column chromatographic method coupled with UV assay was employed for separation of GOTm. The activity was measured by following decrease in abosrbance of NADH at 340 nm. The lowest activity of GOTm determined with a coefficient of variation below 10% was 6 mIU/ml. High GOTm activities were found in acute hepatitis (acute stage), subacute hepatitis and primary biliary cirrhosis and were generally associated with high total GOT (GOTt) activities. The activity ratio of GOTm/GOTt varied depending on the stage and severity of liver diseases. The GOTm/GOTt ratio was decreased in acute, fulminant and subacute hepatitides. No significant reduction in the ratio was found in bile duct obstruction, alcoholic liver injury or metastatic liver cancer. Although relatively high GOTm/GOTt ratios were found in some patients with severe hepatic injury, they had no definite association with poor prognosis. These results indicate that the marked elevation in GOTt over GPT in advanced chronic hepatitis, liver cirrhosis and primary hepatoma was mainly due to preferential leakage of cytoplasmic GOT (GOTs).

KEYWORDS: glutamic-oxaloacetic transaminanse, glutamic-pyruvic transaminase ratio, glutamic-oxaloacetic transaminase isoenzyme, enzyne leakage, liver enzyme, enzyme dedifferentiation, liver diseases

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*PMID: 227231 [PubMed - indexed for MEDLINE]

Acta Med. Okayama **33**, (5), 343—358 (1979)

THE MECHANISM OF RELEASE OF HEPATIC ENZYMES IN VARIOUS LIVER DISEASES
II. ALTERED ACTIVITY RATIOS OF GOT TO GPT IN SERUM AND LIVER OF PATIENTS WITH LIVER DISEASES

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Received March 20, 1979

Abstract. The activities of glutamic-oxaloacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT) and glutamate dehydrogenase (GLD) were determined in liver biopsy specimens and sera of patients with various liver diseases. Mitochondrial and cytosol isozymes of GOT were also separated for their assay. The activity ratio of GOT/GPT in serum was found to reflect the ratio in liver cytosol. The increased ratio in advanced or severe liver diseases, such as liver cirrhosis, was due to the greater decrease in liver cytosol GPT activity, this being pronounced in primary hepatoma. The activity of GLD decreased similarly but less markedly. The relatively greater decrease in GPT compared with GOT in advanced liver diseases was not mainly due to leakage of the enzyme from the liver, but to a specific mechanism associated with hepatic injury or its progression. Other pathological conditions of the liver such as those in obstructive jaundice and alcoholic liver injury also appeared to result in reduced liver GPT activity, which was reflected in the serum as an increased GOT/GPT ratio.

Key words: glutamic-oxaloacetic transaminase/glutamic-pyruvic transaminase ratio, glutamic-oxaloacetic transaminase isoenzyme, enzyme leakage, liver enzyme, enzyme dedifferentiation, liver diseases.

Elevation of serum enzyme activity, particularly of glutamic-oxaloacetic transaminase (GOT : L-aspartate : 2-oxoglutarate aminotransferase, EC 2. 6. 1. 1) and glutamic-pyruvic transaminase (GPT : L-alanine : 2-oxoglutarate aminotransferase, EC 2. 6. 1. 2), has been widely used as an important clinical parameter of hepatic injury to follow the clinical course of liver diseases. Enhanced leakage of these enzymes from damaged hepatocytes appears to be the main cause of increased GOT and GPT activities in the serum of patients with parenchymal liver injury (1, 2). The exact mechanism of this enzyme leakage in hepatic damage is not known. Increased permeability of the plasma membrane of impaired liver cells has been implicated by Henley *et al.* (1) and Takaki (3)

in the enzyme release, although whether this leads to hepatocyte necrosis or not is unknown, despite the demonstration of a significant correlation between the degree of serum enzyme elevation and the extent of liver cell necrosis (4, 5).

Another important factor contributing to the elevated serum enzyme activity is the rate of clearance of leaked enzymes from the blood (6–8). The altered GOT/GPT ratios during the course of acute hepatitis have been explained in part by the difference in half-life between GOT and GPT (1, 7, 9). It is not, however, entirely known whether the low GOT/GPT ratios during the course of acute hepatitis and the high ratio in liver cirrhosis and primary hepatoma can be explained in a similar manner.

In a previous report, the author (10) found that the relatively high activity of GOT over GPT in serum of liver cirrhosis and primary hepatoma was due to increase in both cytoplasmic GOT (GOTs) and mitochondrial GOT (GOTm) activities. Accordingly, the predominant increase in GOT activity in the advanced liver diseases studied did not appear to involve any particular type of hepatocyte injury as to cause preferential mitochondrial damage. In the present study, the activities of GOT and GPT as well as GOTm were determined for liver biopsy specimens and the GOT/GPT ratios in liver were compared with those in serum in an attempt to elucidate the pathophysiological significance of the alteration of GOT/GPT ratio during the course of progression of viral hepatitis to cirrhosis of the liver. This report describes that the change in GOT/GPT ratio in serum reflects the altered hepatic ratio of GOT/GPT, which is intrinsically caused by the disease progression, although enzyme leakage from injured hepatocytes contributes to some extent to the decrease in hepatic enzyme activities (3, 10–12).

MATERIALS AND METHODS

Sera were separated from blood samples withdrawn before breakfast and analyzed for GOT and GPT activities by an SMA-12/60 Technicon Auto Analyzer II (Technicon Instruments Co., Tarrytown, USA) at 37.5°C, unless, otherwise indicated. Liver tissues amounting to 10–30 mg wet weight, were obtained by liver biopsy under peritoneoscopy, and were immediately homogenized 20 times by up and down movement at 1000 r.p.m in 9 volumes of 0.25 M sucrose in a glass homogenizer with a Teflon pestle under an ice-cold condition (13). The debris was discarded and the remaining homogenate was diluted into two aliquots, each amounting to 100–300 μl. One of them received Triton X-100 to give a final concentration of 1%, then both homogenates were centrifuged for one h at 0°C at 25,000×g. The resulting supernatants were diluted with 9 volumes of ice-cold water immediately before the enzyme assay. GOT, GPT and glutamate dehydrogenase (GLD; L-glutamate : NAD oxidoreductase, deaminating EC 1. 4. 1. 2) activities of the diluted supernatants and of some serum specimens were determined by the methods of Karmen *et al.* (14) for GOT and GPT and of

Olson and Anfinsen (15) for GLD by following the rate of decrease in absorbance at 340 nm at 30°C in a final volume of 1 ml. The sample volumes used for GOT, GPT and GLD assays were 50 μ, 50 μl and 100 μl for the liver supernatants and 100 μl, 100 μl and 200 μ for the serum, respectively. The reagents used for GOT and GPT assays were obtained from Boehringer Mannheim Co. (Mannheim, Germany). GOTm was separated by small-scale column chromatography (GOT isozyme test, column method, Nippon Chemiphar Co. Ltd., Tokyo) based on the method employed by Schmidt *et al.* (16). Serum and diluted supernatant were directly applied to the column and GOTm activity was assayed on the eluate within 5 h without loss of activity as is shown in Fig. 1. Tissue enzyme activity was calculated by correcting for the sample dilution and for the recovery of GOTm activity in the column procedure and mean recovery was 78.6% for the supernatant with 1% Triton X-100 and 74.9% for the supernatant without Triton. The total GOTm activity was obtained by summation of the activities recovered with five elutions with no detectable activity in the last one. The recovery was practically the same with samples from different cases and with varying protein concentrations. Mean values for seven different samples are given above. The coefficients of variation were approximately 5% for each.

RESULTS

The enzyme activities determined on biopsied liver, hepatoma and sigmoid cancer metastasized to the liver are listed in Table 1. The activities of GOTs determined with homogenates in the presence of Triton, GOTt (+Triton)-GOTm (+Triton), were comparable to those determined on the separation without Triton, GOTt(−Triton)-GOTm(−Triton) in all the groups. This also applied to GPT, which was mostly confined to the cytoplasmic compartment. The mean activity of total GOT (GOTt) was decreased in the liver of all cases studied as compared with normal controls consisting of non-specific hepatitis, minimum portal fibrosis and Gilbert's disease. The decrease was moderate in acute hepatitis, chronic hepatitis, fatty liver, and marked in alcoholic hepatitis, alcoholic liver cirrhosis and posthepatitic liver cirrhosis. In biliary tract obstruction, no appreciable decrease in GOTt activity was found, whereas there was a considerable decrease in mean GOTt activity in intrahepatic cholestasis. Primary hepatoma had low GOTt activity and metastatic liver cancer from sigmoid cancer had the lowest activity; however in the latter case, postmortem changes could not be excluded. In hepatoma cases, there was no difference in enzyme activities between biopsy and autopsy materials. Considerably reduced GOTt activity was also evident even in tumor-bearing host liver without cirrhosis. The diminished GOTt activities were due to both GOTs and GOTm, the former decrease being less marked except for hepatoma, fulminant hepatitis and metastasized sigmoid cancer to the liver as revealed by the decrease in GOTm/GOTt ratio. This tendency was pronounced in metastatic liver cancer

TABLE 1. ENZYME ACTIVITIES IN THE LIVER AND TUMOR TISSUES IN VARIOUS LIVER DISEASES[a]

Diseases	Enzyme activities (IU/g liver)		
	GOTt	GOTs	GOTm
Normal controls (4)[b]	153.4±46.7	54.5±13.2	103.2±23.3
	65.9±13.7	49.1±10.1	17.2±8.5
Acute hepatitis (11)	117.5±25.1	44.5±7.4	71.6±18.1*
	51.5±6.5*	45.8±22.8	7.0±3.1**
Chronic hepatitis II A (18)	115.1±34.9	47.0±30.6	68.0±17.8**
	51.2±10.2*	42.8±8.6	8.4±2.8**
Chronic hepatitis II B (23)	102.5±43.7	35.0±17.3*	67.6±34.2
	49.6±13.8*	40.7±10.6	8.9±4.3**
Liver cirrhosis (27)	76.6±31.2***	31.0±15.8*	45.4±22.7***
	41.0±15.6**	32.8±13.8*	6.7±3.4***
Alcoholic hepatitis (11)	91.9±26.0**	35.4±9.1 *	56.5±25.2**
	46.2±10.1*	38.8±9.6	7.4±4.7 *
Alcoholic liver cirrhosis (5)	73.3±13.9*	32.0±9.0*	41.3±13.8**
	31.1±4.4**	24.6±5.5**	6.5±2.0
Fatty liver (7)	101.2±30.1	29.6±11.4	71.5±26.0
	41.5±7.8	34.1±7.3	7.3±3.0
Biliary tract obstruction (2)	141.0±4.3	45.8±10.1	95.2±14.4
	66.0±8.5	58.7±8.2	7.3±0.4
Intrahepatic cholestasis (5)	102.6±30.2	47.5±16.1	52.8±19.0
	59.6±22.3	47.6±11.0	9.8±12.5
Primary biliary cirrhosis (1)	175.2	110.2	65.0
	92.2	80.3	11.9
Fulminant hepatitis (2)	26.6±22.2	18.7±16.2	8.0±6.1
	28.8±25.1	22.4±19.6	6.4±5.5
Primary hepatoma (5)	25.2±20.4	18.1±16.2	7.0±8.0
	19.8±16.2	18.1±16.1	1.6±1.3
Hepatoma-bearing tissue (7)	80.2±30.4	50.4±23.3	29.8±20.3
	55.3±22.1	44.0±21.1	11.3±6.2
Tumor (Metastatic liver cancer) (2)	14.0±1.4	8.3±2.3	5.8±0.4
	7.7±3.0	5.8±1.4	2.3±1.6
Tumor-bearing tissue (2)	106.0±13.8	50.8±8.5	55.2±22.2
	73.9±18.4	64.8±16.4	9.1±1.9

The data for the upper lines are from Triton X-100-treated samples and those lower lines from untreated samples. Statistical analysis for significance level was made only for groups more than 10 cases.

a, values are given in mean±standard deviation. *b*, the number of cases studied is shown in the parentheses. The significant levels comparing with the control are *, $P<0.05$; **, $P<0.01$; and ***, $P<0.001$. In all cases of acute hepatitis, the serum GOT activities were below 150 mIU/ml. Chronic hepatitis was classified according to the European classification. The cases of liver cirrhosis were of posthepatitic type. All cases of hepatoma-bearing tissue had liver cirrhosis.

		Activity ratio			
GPT	GLD	GOTt/GPT	GOTs/GPT	GOTm/GOTt(%)	GOTm/GLD
37.4±9.2	103.2±23.3	4.2±1.0	1.8±0.6	62.3±5.6	3.6±1.4
38.5±7.3	17.2±8.5	1.8±0.6	1.5±0.3	24.4±11.9	2.2±1.5
36.5±10.9	13.7±6.8***	3.4±0.9	1.5±0.5	62.0±14.5	6.7±3.6
33.4±13.0	3.5±3.7**	1.8±0.9	1.3±0.5	13.8±6.9	3.4±2.2
30.6±7.7	11.6±5.8***	4.0±1.6	1.6±1.2	61.1±11.8	7.7±3.8
30.7±8.8	4.2±3.7***	1.8±0.6	1.5±0.5	16.3±4.0	4.6±5.0
28.2±11.0	7.7±5.5***	3.8±1.3	1.4±0.8	65.2±12.4	13.1±10.6
26.5±10.0*	2.6±1.9***	2.3±1.2	1.7±0.5	17.3±5.5	4.5±2.5
16.7±8.6***	7.9±5.4***	5.0±1.2	2.1±1.0	58.4±13.2	7.0±5.0
15.4±7.7***	3.1±1.8	3.2±1.8	2.4±0.9	18.2±9.9	3.2±3.5
19.4±9.4**	8.6±5.5***	5.4±2.1	2.2±1.2	59.4±11.2	9.5±6.2
18.7±9.2**	3.0±1.6***	2.9±1.1	2.5±1.0	16.2±8.9	3.5±3.1
9.4±2.2***	5.0±2.6***	8.2±1.8**	3.4±0.5**	55.4±12.7	13.5±10.9
9.8±3.2***	2.2±1.4**	3.6±1.1*	2.8±1.0	21.7±8.2	7.8±7.6
31.0±10.4	7.9±4.4	3.4±0.9	1.0±0.3	69.6±10.4	14.0±10.9
31.9±10.9	2.8±2.0	1.4±0.5	1.2±0.4	17.8±6.7	4.8±3.1
21.5±6.7	13.6±1.4	7.3±2.1	2.5±1.3	67.3±8.2	6.9±0.3
18.0±3.6	0.7±0.2	3.7±1.0	3.5±1.2	11.2±0.9	10.9±2.3
22.7±9.7	9.7±10.2	5.0±1.2	2.5±1.1	51.0±12.1	11.5±9.5
22.7±10.1	4.0±5.9	2.9±0.8	2.5±0.9	13.2±11.7	4.0±2.8
17.2	3.5	10.2	6.4	37.1	18.3
12.4	2.8	7.5	6.5	12.9	4.2
3.4±2.9	0.7±0.4	9.7±1.9	6.1±0.5	36.0±7.3	22.4±20.7
2.4±1.8	0.7±0.4	10.0±2.7	7.8±2.1	22.4±0.3	6.1±5.0
1.6±1.8	0.4±0.2	38.4±53.9	31.5±47.8	32.9±16.5	27.3±19.6
1.3±1.7	0.2±0.1	39.8±58.7	40.6±67.3	21.8±24.0	23.5±32.3
10.6±6.0	2.9±1.6	9.1±4.4	6.2±4.7	37.3±19.8	14.1±11.4
10.1±6.1	1.3±1.0	11.4±14.0	9.3±1.2	21.2±11.3	33.9±48.6
0.8±0.1	0.7±0.1	18.7±4.7	10.9±3.8	42.4±8.5	8.6±2.0
0.6±0	0.6±0.2	14.3±5.9	9.6±2.4	23.3±9.6	3.2±1.2
7.6±3.7	5.4±2.8	17.3±6.6	9.6±5.8	49.8±14.4	11.0±1.7
6.5±3.5	2.0±1.4	13.9±4.6	12.1±4.0	12.5±0.5	7.6±4.3

The three cases (five samples) and the three cases (five samples) in eight cases (12 samples) of primary hepatoma were obtained under autopsy and operation, respectively. The one and the one specimen in fulminant hepatitis were each obtained at autopsy and necropsy. The one and the one case in metastasized sigmoid cancer to the liver were obtained at autopsy and operation, respectively. The one specimen of primary biliary cirrhosis was obtained at operation.

(tumor tissue), primary hepatoma and fulminant hepatitis.

The decrease in activity of GLD was greater than that of GOTm. This occurred in all the liver diseases, particularly in tumor-bearing livers with or without liver cirrhosis, as revealed by decreases in the GLD/GOTm ratio. The activities of these mitochondrial enzymes were detected in the supernatant fraction, and the relative activity of GLD in this fraction, GLD(−Triton)/GLD(+Triton) was generally greater than that of GOTm, GOTm(−Triton)/GOTm(+Triton) in the same fraction except for the control, where the activity of GLD in the supernatant was considerably high and that of GOTm in the supernatant fraction low. The clinical signicance of the elevated mitochondrial enzyme activities in the supernatant fraction could not be assessed, because their possible release from the mitochondria during the process of homogenization could not be excluded. The decrease in GPT activity was greater than that of GOT activity only in advanced or severe liver diseases. It is of great interest that the decrease was even more marked in livers of alcoholic and cholestatic diseases and tumor-bearing host liver and the largest GOTt/GPT or GOTs/GPT ratios were found in hepatoma tissues.

Protein concentrations of liver supernatants are listed in Table 2. Although the concentration of protein in liver tissues decreased in liver cirrhosis, primary biliary cirrhosis and fulminant hepatitis, and even more extensively in hepatic

TABLE 2. PROTEIN CONCENTRATION IN THE LIVER SUPERNATANT FRACTIONS[a]

Diseases	Triton X-100		Diseases	Triton X-100	
	+	−		+	−
Liver cirrhosis (27)[b]	0.866±0.194	0.623±0.148	Tumor[c] (2)	0.453±0.073	0.378±0.048
Acute hepatitis (11)	1.175±0.126	0.783±0.083	Tumor-bearing tissue (2)	0.730±0.195	0.490±0.140
Alcoholic liver injury (16)	0.971±0.193	0.666±0.164	Biliary tract obstruction (2)	1.133±0.123	0.810±0.110
Chronic hepatitis (64)	1.089±0.213	0.770±0.129	Intrahepatic obstruction (5)	1.028±0.174	0.771±0.236
Fatty liver (7)	1.076±0.209	0.747±0.108	Primary biliary cirrhosis (2)	0.773±0.328	0.580±0.200
Primary hepatoma (5)	0.474±0.240	0.359±0.192	Normal controls (4)	1.071±0.287	0.756±0.265
Hepatoma-bearing tissue (7)	0.710±0.394	0.497±0.212	Fulminant hepatitis (2)	0.540±0.040	0.440±0.040

a, The protein concentration is expressed as mg/ml in the ×100 diluted solution of the supernatant fraction. Values are given in mean ± standard deviation.

b, The number of cases studied is shown in the parentheses.

c, The two cases classified in tumor were both obtained from metastasized sigmoid cancer to the liver.

tumors and the host livers, the changes in GOTt, GOTs, GOTm and GLD activities expressed on the basis of liver or tumor tissue protein were essentially the same as those in enzyme activities expressed per unit tissue weight.

The activity ratio of GOTs/GPTs in liver tissue was greater than that in serum in all liver diseases shown in Figs. 2 and 3. In the normal controls, this

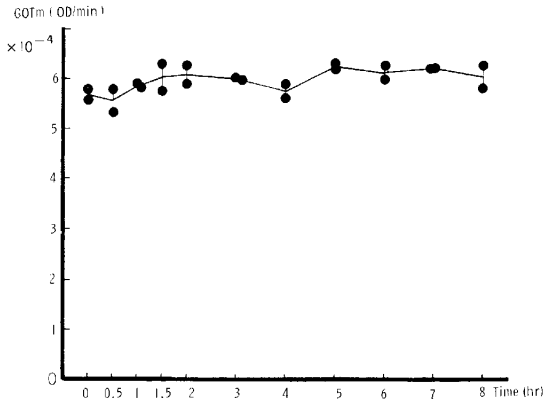

Fig. 1. Stability of liver GOTm in eluate. The eluate from small-scale column chromatography of the supernatant fraction of liver homogenate was kept at room temperature (23°C) and assayed for GOTm activity. Two determinations and their means are shown.

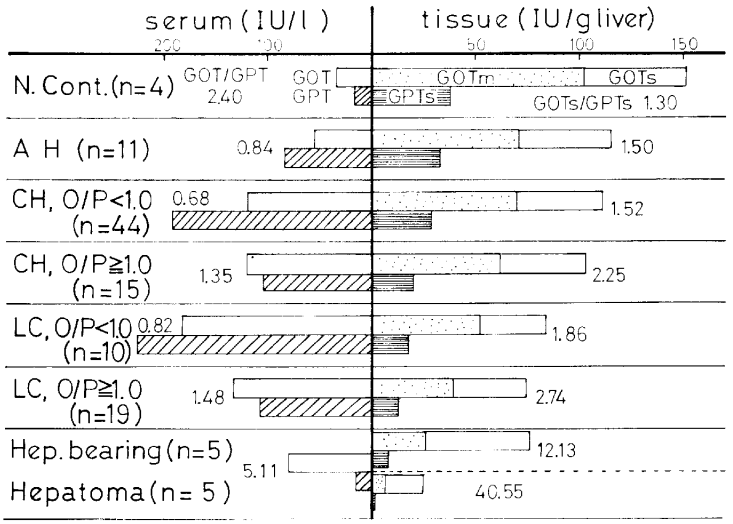

Fig. 2. The GOT, GPT, GOTm, GOTs and GPTs activities in the serum, and in liver and hepatoma tissues. Mean GOTs/GPTs ratios are shown for the given numbers of cases. GOTs and GPTs activities were calculated from the data obtained from the supernatants prepared from the liver homogenates without added Triton. O/P stands for serum GOT/GPT ratio. AH, acute hepatitis; CH, chronic hepatitis; LC, liver cirrhosis; N. Cont., normal controls; and Hep. bearing, hepatoma-bearing liver.

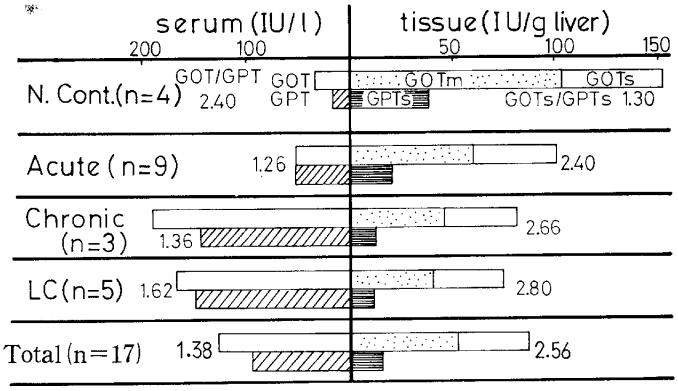

Fig. 3. The GOT, GPT, GOTm, GOTs and GPTs activities in acute and chronic alcoholic hepatitis and alcoholic liver cirrhosis. Acute, acute alcoholic hepatitis; Chronic, chronic alcoholic hepatitis; and LC, alcoholic liver cirrhosis.

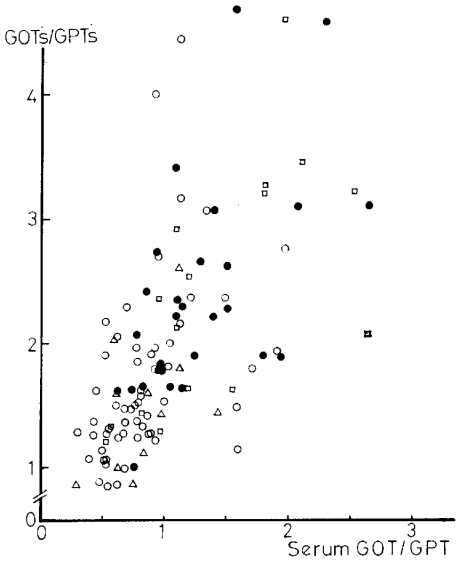

Fig. 4

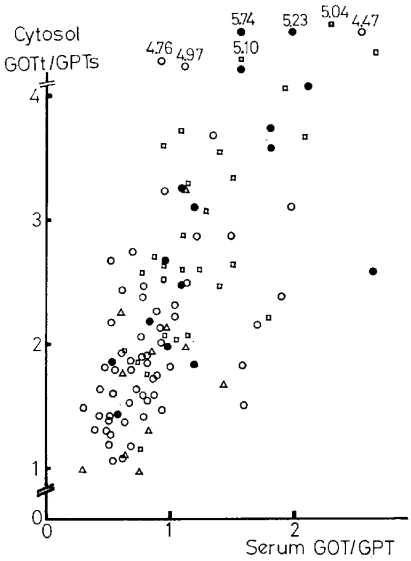

Fig. 5

Fig. 4. The correlation between serum and tissue GOT/GPT ratios. The cytosol GOT/GPT ratio and serum GOT/GPT ratio in △, acute hepatitis; ○, chronic hepatitis; ●, liver cirrhosis; and □, alcoholic liver injury. The coefficient of correlation was $r=0.621$, $P<0.001$.

Fig. 5. The correlation between the cytosol GOTt/GPTs and serum GOT/GPT ratios. The cytosol GOTt was obtained from GOTt activity in the supernatant fraction without Triton. △, acute hepatitis; ○, chronic hepatitis; □, liver cirrhosis; and ●, alcoholic liver injury. $r=0.675$, $p<0.001$.

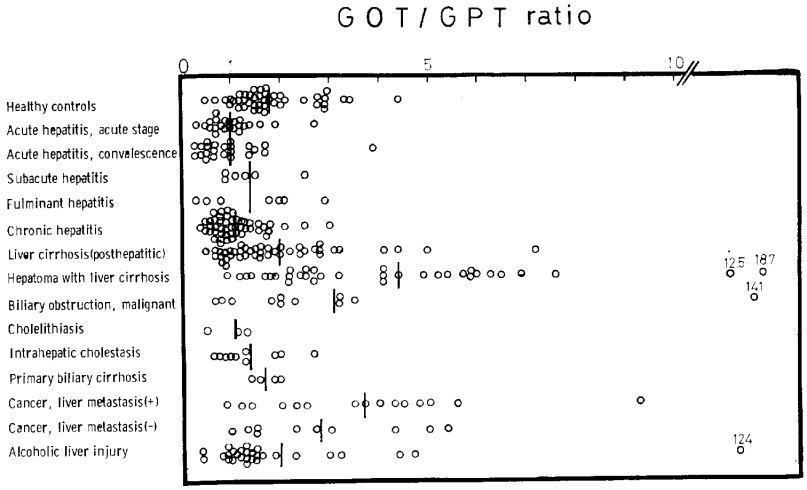

Fig. 6. Serum GOT/GPT ratios in liver diseases. The vertical bar shows the mean of each group.

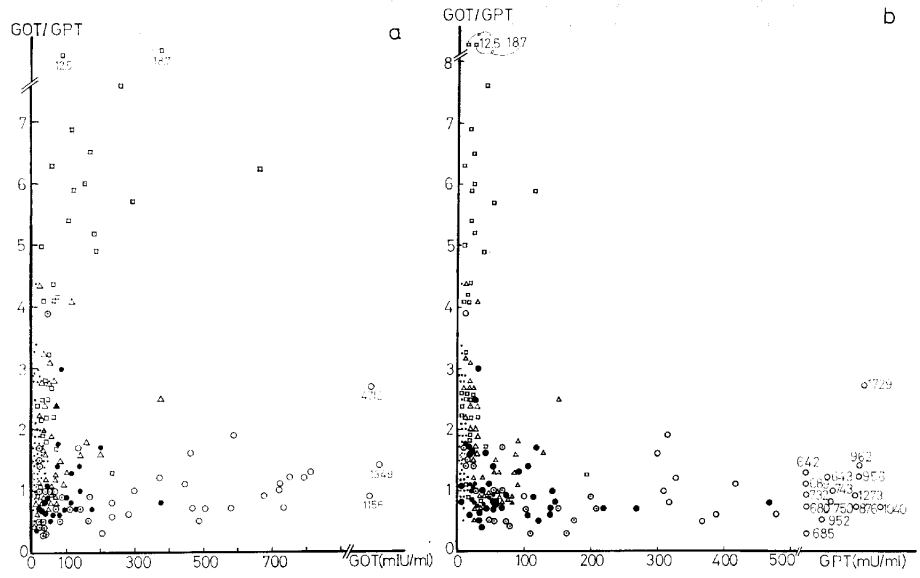

Fig. 7. The serum GOT/GPT ratio as a function of serum GOT or GPT activity. a, GOT/GPT ratios plotted against GOT activities. b, GOT/GPT ratio vs. GPT activities. ·, healthy controls; ○, acute hepatitis, acute stage with GOT more than 150 mIU/ml; ◉, acute hepatitis, convalescent stage; ●, chronic hepatitis; △, liver cirrhosis; and □, primary hepatoma.

relationship was reversed and the serum exhibited a higher ratio. It was also apparent from the same figures that the higher GOTs/GPTs ratio in liver tissue

was reflected in serum as a higher ratio even in the same disease group, such as chronic hepatitis or liver cirrhosis, when each group was divided into two subgroups with serum GOT/GPT ratios greater than or less than 1.0. This was more evident in primary hepatoma, although the extent of the contribution of hepatoma and host liver enzymes to the raised activities of serum enzymes could not be assessed. A similar relationship was also found during the course of alcoholic liver injury. In these groups, however, the GOT/GPT ratio was generally higher both in serum and liver than in virus-induced hepatic injuries. When the GOT/GPT ratio in serum was compared with GOTt/GPTs in liver or hepatoma tissues, the results were essentially identical. The association of increased GOT/GPT ratio in liver tissue with that in serum in liver diseases was also domonstrated by the psesence of a significant correlation between GOTs/GPTs or cytosol GOTt/GPTs ratios, where cytosol GOTt was GOTt activity in the supernatant fraction prepared from the homogenate without Triton. When the cases other than acute and chronic hepatitis, liver cirrhosis

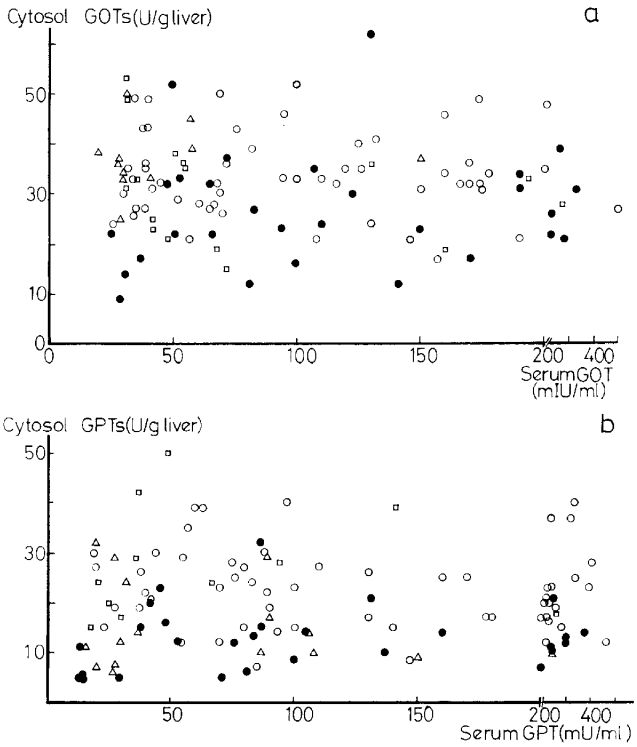

Fig. 8. Enzyme correlation.
a, serum GOT vs. GOTs in the liver.
b, serum GPT vs. GPTs in the liver.

and alcoholic liver injury were also included, a significant correlation of 0.642 was again obtained (Figs. 4 and 5).

Since the serum GOT/GPT ratio changed, depending not only on the type of hepatic injuries involved (Fig. 6) but also the serum level of GOT or GPT, the GOT/GPT ratio was plotted against the absolute GOT or GPT activity

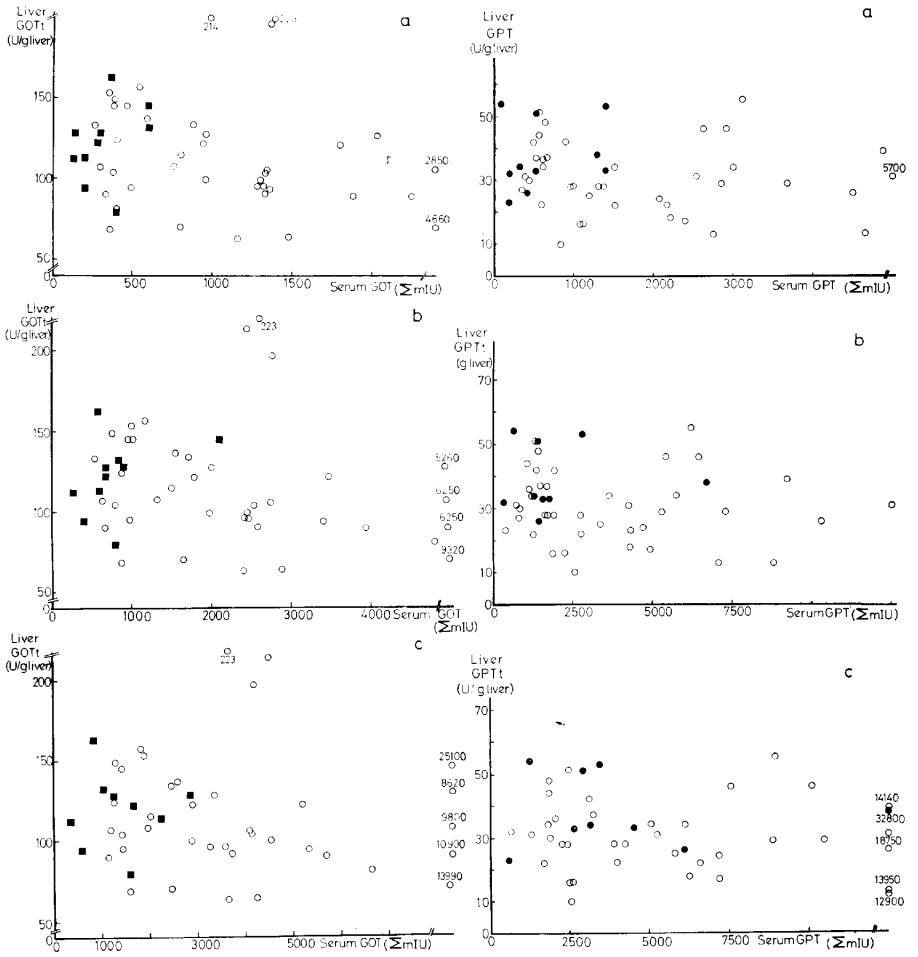

Fig. 9 **Fig 10**

Fig. 9. Liver GOTt activities compared with the summation of the serum GOT activities in acute (● or ■) and chronic hepatitis (○). a, summation of serum GOT activities for 10 days before liver enzyme analysis ($r=-0.264$, $p>0.05$); b, 20 days before ($r=-0.244$, $p>0.05$); and c, 30 days ($r=0.033$, $p>0.05$).

Fig. 10. Activities compared with the summation of the serum GPT activities in acute and chronic hepatitides. Abbreviations used, see the legend to Fig. 8 a, $r=-0.161$, $p>0.05$; b, $r=-0.135$, $p>0.05$; and c, $r=-0.013$, $p>0.05$.

Fig. 7). The groups of acute hepatitis and liver cirrhosis with or without hepatoma were clearly separated. The cases with GOT/GPT ratios above 5 were all primary hepatoma in spite of normal GPT activities. The lower ratio of GOT/GPT in acute hepatitis was also evident even if the increase in GOT or GPT activity was moderate. Thus, the comparisons made in Figs. 3–5 between serum and tissue GOT/GPT ratio without referring to the absolute serum GOT or GPT activity were considered valid. When the serum GOT and GPT activities determined at the time of tissue enzyme analysis were compared with the GOT and GPT activities in the liver supernatant (Fig. 8), respectively, no discrete correlation was found between them, indicating that these enzyme activities do not change by an interrelated mechanism. In other words, the increased serum GOT or GPT level does not necessarily accompany the reduction in liver GOT or GPT activity as a direct consequence of enzyme leakage. However, when a more precise comparative study was made by taking the sum of serum GOT or GPT activity within a particular period of time, $\sum_{day=1}^{10}$ mIU/ml/day $=\sum_{day=1}^{10}$mIU, $\sum_{day=1}^{20}$ and $\sum_{day=1}^{30}$ as the leaked total enzyme activity during the indicated period of time before tissue enzyme analysis, a weak ($p>0.05$) but negative correlation was found between the serum GOT and GPT and liver GOT and GPT respectively, for the preceding 10 and 20 days, and no correlation was obtained for the third 10 days (Figs. 9 and 10). The results confirmed the generally accepted mechanism that leakage of GOT or GPT from the liver leads to the reduced tissue activities of these enzymes, although this mechanism appeared to play a minor role as long as the maximum increase in serum GOT or GPT activity was no more than 32,800 mIU/30 days.

DISCUSSION

Several mechanisms, by which the serum activities of GOT and GPT increase in liver diseases, have been proposed since the initial observation made by Wróblewski and LaDue (17). Among them are: 1) increased permeability of hepatocyte membrane to GOT and GPT caused by hepatocyte injury (1, 2, 18), 2) decreased clearance rate for GOT and GPT (19–21) and 3) the presence of activator in the blood (22, 23).

The importance of the difference in clearance rate of leaked enzymes in explaining altered GOT/GPT ratios in different stages of hepatitis has been suggested by several workers (7, 8, 24). The activation of GPT during enzyme leakage from the hepatocyte appears to lower the GOT/GPT ratio in serum (25). The possibility that the altered tissue levels of GOT and GPT are also reflected in the serum has also been postulated without convincing evidence. Generally, the

tissue levels of GOT and GPT are considered to decrease as a result of the leakage into the blood following hepatic injury, based on the results that a mirror image exists between serum and liver enzyme activities in acute liver damage in clinical cases and experimental animals (11, 12). This is true when the mean activities of GOT and GPT in serum and liver in different liver diseases or during the course of acute liver injury are compared (3, 12, 18). However, no consistent relationship between the liver and serum enzyme activities was found when the values of individual cases were compared at the time of liver enzyme assay. This is understandable, since the change in serum levels of GOT or GPT is rapid and single determinations on serum at the time of hepatic enzyme analysis might miss any preceding rise in serum enzyme level. In fact, the summation of serum GOT or GPT activities for 10 to 20 days preceding the liver enzyme analysis had weak but negative correlation with the liver enzyme activities. Accordingly, the elevated serum activity of GOT or GPT, when persisted for a certain period of time, appeared to reduce the liver activities of these enzymes. However, the decrease in liver enzyme activities, particularly GPT, was much greater in liver cirrhosis, where the serum GPT elevation was relatively small. Therefore, the major factor causing the decrease in activity of GPT in advanced liver diseases could not be leakage of GPT from the liver. Hepatic injury *per se* also appears to play an important role in reducing the activities of these enzymes, particularly GPT, in severe or advanced liver diseases. This is in accord with the results obtained for other hepatic enzymes, such as glucokinase, glucose 6-phosphatase, fructose 1, 6-diphosphatase and pyruvate kinase-Type L (26, 27), a group of enzymes considered to be more differentiated or liver-specific. Similarity of the enzyme patterns in injured livers and hepatoma tissues has also been shown for the carbohydrate-metabolizing enzymes by Taketa *et al.* (26). This would also apply to GOT and GPT; the latter is relatively more specific to liver; therefore, decrease in hepatic injury was more pronounced, and extreme reduction in liver GPT activity was found in hepatocellular carcinoma (28, 29).

The important results emerging from this study are that the increased ratio of GOT/GPT in serum reflects the diminished activity of liver GPT and is a good measure of the enzyme dedifferentiation in the liver caused by hepatic injury as proposed by Taketa *et al.* (26, 30, 31). It is also noteworthy that the serum GOT/GPT ratios in various liver diseases were slightly greater than those of liver cytosol. Accordingly, the serum GOT/GPT ratio could be regarded as representing the cytosol GOT/GPT ratio.

It is of some interest that among benign liver diseases the highest GOT/GPT ratio was found in liver cirrhosis, which could be considered as a preneoplastic state in the sense that hepatocellular carcinoma develops frequently in

liver cirrhosis. However, hepatocytes with an undifferentiated enzyme pattern or with other cancer-marker protein, such as α-fetoprotein, are not necessarily the precursor cells of hepatocellular carcinoma (27). Therefore, the high GOT/GPT ratio found in liver cirrhosis is only significant in terms of the increased possibility of developing hepatoma. It is also noteworthy that the patients with liver cirrhosis and a GOT/GPT ratio above five are highly likely to have hepatoma.

Another important result was that higher GOT/GPT ratio found in sera from patients with cholestasis (intrahepatic and extrahepatic) and alcoholic liver injury was associated with decreased GPT activity in the liver. This could be interpreted as indicating that cholestasis and alcoholic liver injury specifically lower GPT activity in the liver by some mechanism opposite to those inducing increased alkaline phosphatase, leucine aminopeptidase and γ-glutamyltranspeptidase activities (32–34).

Acknowledgment. The author wishes to express his profound thanks to President Kiyowo Kosaka at Okayama University (former Professor of First Department of Internal Medicine) and Professor Hideo Nagashima for their interest and support, to Dr. Kazuhisa Taketa for his accurate instruction, to Dr. Masahiro Kubota and his group for their active collaboration through liver biopsy under peritoneoscopy.

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